

HCM-associated *ALMS1* variant: Allele drop-out and frequency in Italian Sphynx cats

Abstract

Hypertrophic cardiomyopathy (HCM) is the most common cardiomyopathy in domestic cats, and some inherited variants are available for genetic testing. A variant of the Alstrom syndrome protein 1 gene (*ALMS1*) was recently reported to be associated with HCM in the Sphynx cat breed (A3: g.92439157G>C). Genetic screening of the variant, promoted by the Osservatorio Veterinario Italiano Cardiopatie and Genefast Laboratory, was offered to Sphynx cat owners and breeders in Italy. Genotype data were initially obtained by Sanger sequencing. In one case where the samples of a trio were available, inconsistency in the vertical transmission of the variant suggested an allele dropout (ADO) of the wt allele. A new external primer pair was designed as an alternative to the original. The larger PCR product obtained was sanger sequenced, and five novel single nucleotide variants (SNVs) not yet annotated in open-access databases were detected. Three of these SNVs were within the original primer-binding regions and were assumed to have caused ADO. The haplotype, including the ADO SNVs, was detected in two cats belonging to different lineages. To accurately genotype *ALMS1* g.92439157G>C in the samples, we set up a real-time TaqMan MGB assay while avoiding all surrounding SNVs. At g.92439157G>C, for 136 Sphynx cats, g.92439157 C variant was highly widespread (freq. >0.50). The present study reports five new variants surrounding *ALMS1* g.92439157G>C that must be considered when designing the test. The study also indicates the need to verify the correspondence between the g.92439157 C variant frequency and the prevalence of HCM by increasing clinical visits and follow-ups and finally to promote genetic counselling for accurate management of mating plans in Italian Sphynx cats.

Hypertrophic cardiomyopathy (HCM) is the most common cardiomyopathy in domestic cats. In pure breeds and randomly bred cats, causative variants of HCM have been identified in different genes (Meurs et al., 2005,

2007; Schipper et al., 2019) and diagnostic genetic tests are widely used to help practitioners with prognosis and breeders with selection. Recently, a single nucleotide variant (SNV) in the Alstrom syndrome protein 1 gene (*ALMS1*) was reported to be associated with HCM in the Sphynx cat breed, resulting in an immediate request for testing by veterinarians, owners and breeders. The variant is a G>C substitution in *ALMS1* exon 12 at the feline genome position A3: 92439157 (ENSFCAG00000008756, vs.9), which is predicted to change glycine to arginine (Meurs et al., 2021).

In 2021, 43 male and 69 female Sphynx cats were registered with the Associazione Nazionale Felina Italiana, the biggest fancy cat association in Italy. Soon after the identification of the *ALMS1* variant, the authors, via the Osservatorio Veterinario Italiano Cardiopatie (OVIC) and partner laboratory Genefast, promoted genetic screening of the variant at the national level for owners and breeders. Samples were also collected from customers who were not affiliated with the OVIC and did not request echocardiography. Primers *ALMS1_F* and *ALMS1_R* were designed based on the *Felis catus* genomic sequence vs.9 (Buckley et al., 2020) to obtain a 157 bp amplicon and genotyping was performed using Sanger sequencing (Figure 1).

An inconsistency between a homozygous variant sire and his homozygous wt offspring was reported by a breeder. The breeder provided a blood sample from the dam that tested heterozygous. After confirming the parental relationships using the ISAG STR panel, allele dropout (ADO) of the wt allele in the father was hypothesised. A new set of PCR primers were then designed, covering the surrounding region of the *ALMS1* variant and the original PCR primer sites. The new primers (*ALMS1_F2* and *ALMS1_R2*) generated a 306 bp amplicon (Figure 1; PCR protocol in Table S1). Using 19 selected samples, including the trio and 12 g.92439157G>C variant homozygotes, redesigned PCRs and Sanger sequencing were performed. Within the trio, the sire was heterozygous, confirming that a wt allele dropout had previously occurred when the original PCR assay was used. By aligning all longer sequences, five SNVs

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Authors. *Animal Genetics* published by John Wiley & Sons Ltd on behalf of Stichting International Foundation for Animal Genetics.

ALMS1_F TCTCACTTTGCTGTAGGATCCA ALMS1_R CCACTGAGAAGGACGGCTTA
 F.catus_9.0 pos. A3: 92439046-92439202 short amplicon (157bp) - original

ALMS1_F2 TCCCCTTCTGATCACACTGC; ALMS1_R2 CCTGCATTATCTTTCTGAGGGG
 F.catus_9.0 pos. A3: 92438928 – 92439233 long amplicon (306bp) - redesigned

tccccttctgatcacactgcaaacactcctattgtatcttttaataagaagcgggttaagaggtaagtttttgtctctg
ttcaacttcatgtctatatatttgtca[g/a]ttgtactactttc[t/c]cacttttgcgtg[t/a]aggatccaatgatg
 ccacgcctccagacttcccagctgaggtgctaggcaccaggatgatga[c/t]ctgtccatccctgctgacactaac
 ataaagaa[g/c]ggatctacagtaagagggccgtggctaaqcc[g/a]tccttctcagtggggaaaaacccccctcag
aaagataatgcagg

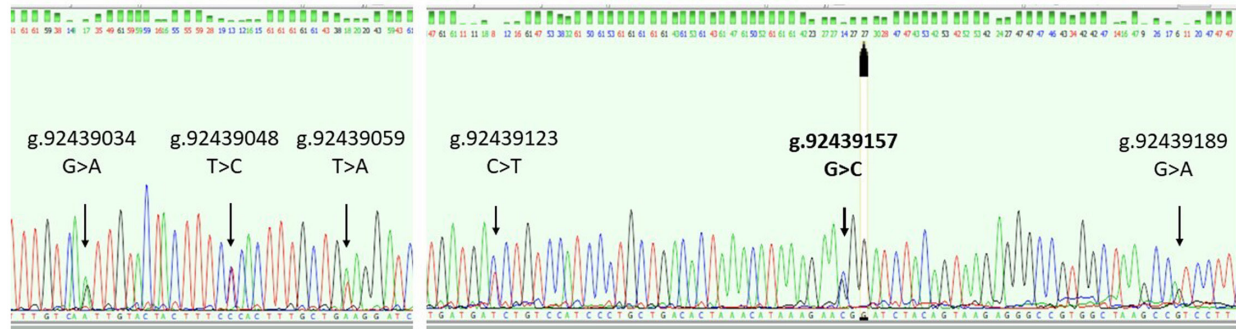


FIGURE 1 Top: *ALMS1* test, large amplicon sequence on *Felis catus* vs. 9.0 (Buckley et al., 2020). The intronic regions are in italics, the pair of primers for both redesigned (large) and original (short) PCR is underlined, variations detected by Sanger sequencing are in brackets, and the *ALMS1* variation published by Meurs et al. (2021) is in bold. Bottom: *ALMS1* large amplicon sequences on *F. catus* vs. 9.0. Electropherograms from two different cats showing the positions of all the SNVs.

other than the published g.92439157G>C that were not already annotated in public databases were detected: g.92439034G>A; g.92439048T>C; g.92439059T>A; g.92439123C>T; and g.92439189G>A (Figure 1). Two (g.92439048T>C and g.92439059T>A) were in the forward (*ALMS1_F*) and one (g.92439189G>A) was in the reverse (*ALMS1_R*) primer site of the original short PCR; these are very probably the cause of ADO in the sire (Figure 1). Among the cats originally genotyped as homozygous for the g.92439157 C variant, there was another cat in addition to the sire of the trio that yielded a heterozygous result with the new primer set. Importantly, the two cats with ADO were not related based on their pedigree.

The five novel SNVs were either intronic substitution – g.92439034G>A, g.92439048T>C and g.92439059T>A – or exonic synonymous substitution – g.92439123C>T and g.92439189G>A (Figure 1). Two intronic SNV (g.92439048T>C and g.92439059T>A) were clustered near the splicing site but were not likely to cause alternative splicing.

Haplotypes were inferred using chromatograms of either the wt or var homozygote samples at the g.92439157G>C position and trio samples. Three different haplotypes were identified: GTTTCG associated with the *ALMS1* g.92439157 C variant (underlined) and ACTCGG and ACACGA, both associated with the g.92439157G wt nucleotide (underlined); the ACACGA haplotype was recorded in the ADO cases (Table S2). Excluding the offspring in the trio, in 18 unrelated samples 100% of the g.92439157 C alleles showed the GTTTCG haplotype, whereas the g.92439157G variant

was associated 75% with the ACTCGG haplotype and 25% with the ACACGA haplotype.

To accurately genotype the g.92439157G>C variation in all samples, avoiding all SNVs in the flanking regions, a real-time TaqMan MGB assay with primers F-sphynx CCATCCCTGCTGACACTAAACAT and R-sphynx GGTTTTCCCCTACTGAGAAGGA and G allele probe (wt) VIC-CTTACTGTAGATCCCTTCTTT-NFQ and C allele probe (variant) FAM-CTGTAGATCCGTTTCTTT-NFQ was designed. The PCR mix contained 1× TaqManUniversal PCR Master Mix (Thermo Fisher), 1× Custom Taqman SNP Genotyping Assay (Thermo Fisher), 2.5 µl of DNA template, and molecular biology-grade water to a final volume of 25 µl. Each sample was assayed in duplicate and no DNA control was assayed in each run. The assay was validated using all the collected samples.

Of the 136 Sphynx cat sampled, with or without clinical data, the genotype frequencies were distributed as follows: 33 wt/wt (24.3%), 67 wt/var (49.3%) and 36 var/var (26.5%), and the var allele frequency was 51.1%. Considering only the younger cats ($n = 59$, ≤4 years old), the genotypes were distributed as follows: 18 wt/wt (30.5%), 27 wt/var (45.8%) and 14 var/var (23.7%), and a var allele frequency of 46.6%, showing widespread presence of the variant in the younger generations of pet and breeding cats.

In the last decade, the number of genetic variants identified as causative of diseases has greatly increased in pets, and diagnostic tests are now widely used, helping practitioners in diagnosis and prognosis and breeders

in selection. Good interactions between owners, breeders, breeders' associations, practitioners, researchers and service laboratories (as in OVIC) can be functional and beneficial for monitoring genetic frequencies and disease prevalence in a region. Allele dropout artefacts are caused by occasional amplification failure of one of the two alleles at a given genomic DNA locus. It represents a well-known risk of misdiagnosis, particularly for amplification-based methods (0.44% occurrence of genotype mistypings in humans) (Blais et al., 2015) and reduces the diagnostic yield of genetic testing in cardiomyopathies (Shestak et al., 2021). However, in humans within PCR failure cases systematic allele-specific failures owing to sequence variants occur in only 6% of cases (95% CI, 3%–11%) (Blais et al., 2015). Of these, 75% variants were in the primer-binding sites, whereas the remaining were located within the amplicon (Blais et al., 2015). However, when comparing the percentages, it should be noted that the studies and annotations available are still more extensive for humans than for cats. In the present study, by re-genotyping 12 g.92439157 C homozygotes, we detected SNVs in the binding sites of the original primer pair set, identifying a non-random ADO of the wt allele in two unrelated cats. If the ADO was mainly due to the absence of annotation of those variants into public variant repositories, which misled the first genotyping design, the high proportion of ADO in such a small sample could be motivated by high inbreeding and thus genetic similarity in the Sphynx population. The authors did not find evidence to explain why the two ADO cases, which also shared the ACACGA haplotype, had no kinship ties based on their pedigree. This could have been due to misregistration or the presence of a common ancestor several generations prior. Despite having solved with a new PCR design the risk of ADO of the wt allele and to overestimate the variant allele, the authors recorded a percentage of the g.92439157 C variant above 45%, which is high for a causative variant, even considering possible inflation by some unavoidable collection bias. Echocardiographic controls were implemented by the Osservatorio, and 75.3% of the healthy Sphynx cats that underwent echocardiography showed the g.92439157 C variant in a single or double dose. However, these controls do not permit the evaluation of the effect of the g.92439157 C variant on the disease in the Italian Sphynx population, owing to low numerosity and the fact that only a relatively small number of cats had reached an age of approximately 4 years, at which one can be definitively confident in the phenotype classification.

Overall, the present data indicate that care must be taken with good practice (CLSI, 2012) when typing the *ALMSI* g.92439157 C variant. The authors suggest the need for close genetic counselling of the breeders, to avoid dramatic reduction of the genetic pool owing to uncontrolled genetic testing and mating exclusion,

and the need to improve clinical controls to monitor the effect of the variant on the health of the Italian population.

KEYWORDS

allele drop-out, *ALMSI* SNV, HCM, Sphynx cat, variant frequency

ACKNOWLEDGEMENTS



The authors thank Animal Bio Arkivi at the University of Milan, all of the specialists of the Osservatorio Veterinario Italiano Cardiopatie, the Associazione Nazionale Felina Italiana Studbook, Mr Vincenzo Macri, and the Italian Sphynx breeders and owners for fruitful collaboration.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The five SNVs identified in this study have been deposited in the European Variation Archive (Cezard et al., 2021) at EMBL-EBI under accession no. PRJEB60498 (<https://www.ebi.ac.uk/eva/?eva-study=PRJEB60498>).

Maria Elena Turba¹
Paolo Ferrari^{2,3}
Raffaella Milanese⁴
Fabio Gentilini⁵ 
Maria Longeri⁴ 

¹*Genefast srl, Forlì, Italy*

²*Osservatorio Veterinario Italiano Cardiopatie, Azzano San Paolo, Italy*

³*Clinica Veterinaria Orobica Anicura Via Zanica, Bergamo, Italy*

⁴*Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy*

⁵*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Italy*

Correspondence

Maria Longeri, Department of Veterinary Medicine and Animal Science, University of Milan, 26900 Lodi, Italy.
Email: maria.longeri@unimi.it

ORCID

Fabio Gentilini  <https://orcid.org/0000-0002-0970-4827>
Maria Longeri  <https://orcid.org/0000-0001-7863-1855>

REFERENCES

- Blais, J., Lavoie, S.B., Giroux, S., Bussi eres, J., Lindsay, C., Dionne, J. et al. (2015) Risk of misdiagnosis due to allele dropout and false-positive PCR artifacts in molecular diagnostics: analysis of 30,769 genotypes. *The Journal of Molecular Diagnostics*,

- 17, 505–514. Available from: <https://doi.org/10.1016/j.jmoldx.2015.04.004>
- Buckley, R.M., Davis, B.W., Brashear, W.A., Farias, F.H.G., Kuroki, K., Graves, T. et al. (2020) A new domestic cat genome assembly based on long sequence reads empowers feline genomic medicine and identifies a novel gene for dwarfism. *PLoS Genetics*, 16(10), e1008926. Available from: <https://doi.org/10.1371/journal.pgen.1008926>
- Cezard, T., Cunningham, F., Hunt, S.E., Koylass, B., Kumar, N., Saunders, G. et al. (2021) The European Variation Archive: a FAIR resource of genomic variation for all species. *Nucleic Acids Research*, 28, gkab960. Available from: <https://doi.org/10.1093/nar/gkab960>
- CLSI. (2012) *Quality management for molecular genetic testing; approved guideline. CLSI document MM20A*. Wayne, PA: Clinical and Laboratory Standards Institute.
- Meurs, K.M., Norgard, M.M., Ederer, M.M., Hendrix, K.P. & Kittleson, M.D. (2007) A substitution mutation in the myosin binding protein C gene in ragdoll hypertrophic cardiomyopathy. *Genomics*, 90, 261–264. Available from: <https://doi.org/10.1016/j.ygeno.2007.04.007>
- Meurs, K.M., Sanchez, X., David, R.M., Bowles, N.E., Towbin, J.A., Reiser, P.J. et al. (2005) A cardiac myosin binding protein C mutation in the Maine Coon cat with familial hypertrophic cardiomyopathy. *Human Molecular Genetics*, 14, 3587–3593. Available from: <https://doi.org/10.1093/hmg/ddi386>
- Meurs, K.M., Williams, B.G., DeProspero, D., Friedenber, S.G., Malarkey, D.E., Ezzell, J.A. et al. (2021) A deleterious mutation in the ALMS1 gene in a naturally occurring model of hypertrophic cardiomyopathy in the Sphynx cat. *Orphanet Journal of Rare Diseases*, 16(1), 108. Available from: <https://doi.org/10.1186/s13023-021-01740-5>
- Schipper, T., Van Poucke, M., Sonck, L., Smets, P., Ducatelle, R., Broeckx, B.J.G. et al. (2019) A feline orthologue of the human MYH7 c.5647G>A (p.(Glu1883Lys)) variant causes hypertrophic cardiomyopathy in a domestic shorthair cat. *European Journal of Human Genetics*, 27(11), 1724–1730. Available from: <https://doi.org/10.1038/s41431-019-0431-4>
- Shestak, A.G., Bukaeva, A.A., Saber, S. & Zaklyazminskaya, E.V. (2021) Allelic dropout is a common phenomenon that reduces the diagnostic yield of PCR-based sequencing of targeted gene panels. *Frontiers in Genetics*, 1(12), 620337. Available from: <https://doi.org/10.3389/fgene.2021.620337>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.